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## A single social stress-experience alters glutamate receptor-binding in rat hippocampal CA3 area

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The distribution of glutamate receptors in the rat hippocampus was investigated 24 h after the social stress of confrontation with a dominant opponent. AMPA-type glutamate receptors were labeled with the antagonist [<sup>3</sup>H]CNQX, and NMDA-type receptors were labeled with the competitive antagonist [<sup>3</sup>H]CGP39653. Increased [<sup>3</sup>H]CGP39653 labeling was exclusively found in the CA3 stratum radiatum and a decreased [<sup>3</sup>H]CNQX labeling was found in several hippocampal areas. Consequently, the ratio NMDA/AMPA binding was significantly increased in CA3 stratum oriens and CA3 stratum radiatum. These results suggest that a single unescapable social stress of defeat alters the impact of the excitatory neurotransmitter glutamate, which is restricted to hippocampal CA3 neurons. Possible consequences of the present findings are discussed.

Hippocampal neurons in the CA3 and CA4 area are vulnerable to effects of acute and long-term stress [11, 16, 17, 23, 24]. Long-term corticosterone treatment results in similar effects [1, 11, 16, 24, 25]. It has been hypothesized that the glutamatergic mossy fiber input on corticosteroid receptor containing cells contributes to this effect [11]. In addition, several stressors alter hippocampal neuronal plasticity assessed by studying glutamate mediated electrophysiological phenomena like long-term potentiation [4, 19, 20] and kindling [2], and synaptic efficacy in the entorhinal-dentate pathway [7].

Glutamate, the primary excitatory neurotransmitter in the hippocampus, mediates both normal transmission and excitotoxic effects [6, 12, 18]. At least three major, pharmacologically distinct, ionotropic receptors mediate the excitatory postsynaptic actions of glutamate [3, 13]. The receptor subtypes are named according to their selective agonists as the *N*-methyl-D-aspartate (NMDA), the  $\alpha$ -amino-3-hydroxy-*S*-methyl-4-isoxazolepropionic acid (AMPA) and the kainate receptor. Much attention has focused on the function of AMPA- and NMDA-type receptors. AMPA-type receptors are thought to be involved in the fast EPSPs, whereas the NMDA receptor activation is voltage dependent (e.g. evoked by AMPA-

kainate receptor stimulation) and consequently involved in long-term physiological changes [14].

In addition to the effects of daily stress and corticosterone treatment, prolonged social stress has been associated with hippocampal atrophy in baboons [22]. This might indicate that social conditions can affect hippocampal function, presumably through alterations in glutamatergic neurotransmission. Here we report alterations in rat hippocampal AMPA- and NMDA-receptor binding 24 h after a single unescapable social stress of confrontation with a dominant opponent.

Male Tryon Maze Dull (TMD S3) rats of 4 months of age (originally derived from Cpb, TNO Zeist and locally bred at the Department of Animal Physiology, University of Groningen, The Netherlands) were housed socially (6–7 animals per cage) on a 12 h light–dark schedule (7:00–19:00 dark). Experiments were performed during the dark period. Six rats were confronted with a dominant trained fighter TMD S3 rat. The confrontation took place in a wooden cage (85 × 60 × 50 cm) which was permanently occupied by the dominant rat. The social interaction was by definition ended by display of submissive behavior of the experimental animal. After display of totally submissive behavior, the experimental rat was put into a small wire mesh cage and replaced in the cage of the dominant rat for 1.5 h. This was done to prevent direct physical interaction, but to allow psychosocial interaction between the defeated experimental rat and the

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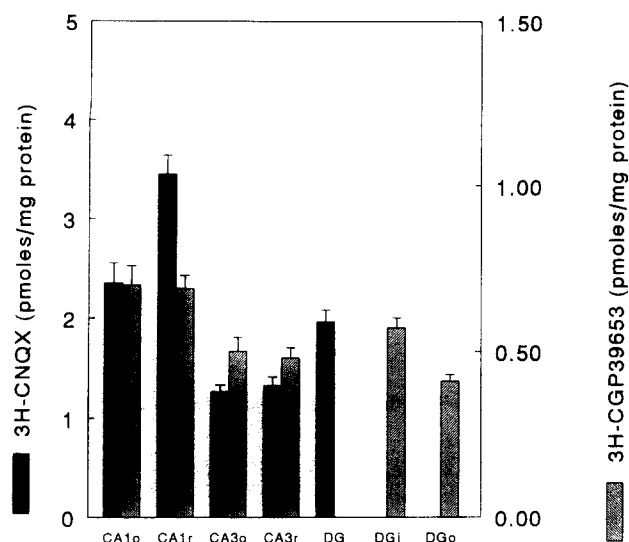


Fig. 1.  $^3\text{H}$ ]CNQX and  $^3\text{H}$ ]CGP39653 binding in different regions of the dorsal hippocampus in control rats. Optical density of autoradiograms was measured using IBAS semi-automized imaging system and converted into pmol/mg protein. CA1 = stratum oriens CA1, CA1r = stratum radiatum CA1, CA3o = stratum oriens CA3, CA3r = stratum radiatum CA3, DG = molecular layer of the dentate gyrus, DGi = inner molecular layer of the dentate gyrus, DGo = molecular layer of the dentate gyrus.

dominant rat. Six control rats were allowed to have 1.5 h psychosocial interaction with the dominant rat while housed in the wire mesh cage. After 1.5 h the experimental and control rats were housed individually in plexiglas cages (25 × 25 × 30 cm) and kept on the same light-dark schedule until decapitation 24 h later.

Following decapitation, the brains were rapidly removed and frozen on dry ice. Sections of 15  $\mu\text{m}$  were cut at  $-15^\circ\text{C}$ , thawmounted on gelatin-coated slides and dried overnight at  $4^\circ\text{C}$ . Adjoining sections of 15  $\mu\text{m}$  were used for acetylcholine esterase staining to reveal distinct areas. Sections from the dorsal hippocampus were used for receptor labeling. Six sections from each animal were taken for total binding, whereas two sections were used for non-specific binding. To label the AMPA receptor, sections were preincubated in 50 mM Tris-HCl buffer (pH 7.4) 3 times for 15 min at room temperature. Preincubation was followed by incubation for 45 min in 300  $\mu\text{l}$  of 50 mM Tris-HCl buffer (pH 7.4) containing 100 nM of the antagonist  $^3\text{H}$ ]CNQX (New England Nuclear, 15.6 Ci/mmol). Non-specific binding was determined by adding 10 mM L-glutamate. Following incubation the slides were rinsed 3 times 5 s in 50 mM Tris-HCl buffer (pH 7.4), dipped into bidest and air dried. NMDA receptor autoradiography was performed as previously described [8]. To label the NMDA receptor, sections were preincubated in 50 mM Tris-HCl buffer (pH 8.0) 3 times for 15 min at room temperature and subsequently incu-

bated for 60 min in 300  $\mu\text{l}$  of 50 mM Tris-HCl buffer (pH 8.0), containing 50 nM of the competitive antagonist  $^3\text{H}$ ]CGP39653 (New England Nuclear, 37.2 Ci/mmol), glutamate dehydrogenase (Boehringer Mannheim), NAD (Boehringer Mannheim) and hydrazine (Sigma, St. Louis, MO). Non-specific binding was determined by adding 10 mM 2-amino-5-phosphonopentanoic acid (APV). Following the incubation, the slides were rinsed 3 times 30 s in 50 mM Tris-HCl buffer (pH 8.0) buffer dipped into distilled water and air dried. Slides were dried overnight at  $4^\circ\text{C}$ . Sections and radioactivity standards (Amersham, UK) were covered with autoradiographic film (Hyperfilm, Amersham, IL) and exposed for 6 days ( $^3\text{H}$ ]CNQX) or 4 weeks ( $^3\text{H}$ ]CGP39653) at  $4^\circ\text{C}$ . After exposure, films were developed for 4 min (Ilford

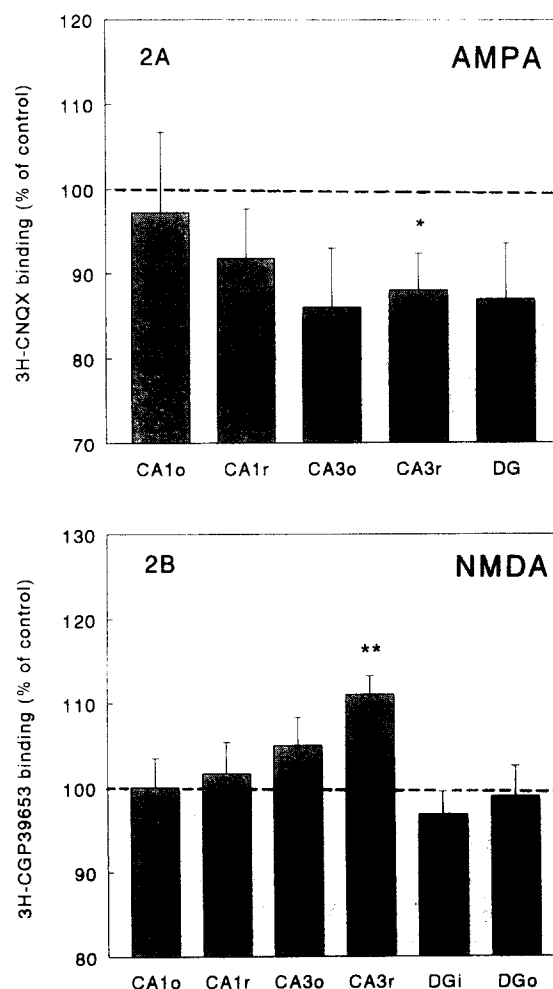


Fig. 2. Effect of social defeat on AMPA (A) and NMDA (B) receptor binding in rat dorsal hippocampus. NMDA and AMPA receptors were labeled using  $^3\text{H}$ ]CGP39653 and  $^3\text{H}$ ]CNQX respectively. Data are expressed as percentage of controls. \* \*\* Indicate significantly altered binding at the 0.10 and 0.05 levels, respectively. CA1o = stratum oriens CA1, CA1r = stratum radiatum CA1, CA3o = stratum oriens CA3, CA3r = stratum radiatum CA3, DG = molecular layer dentate gyrus, DGi = inner molecular layer of the dentate gyrus, DGo = outer molecular layer of the dentate gyrus.

TABLE I

RATIO OF NMDA AND AMPA BINDING IN THE DORSAL HIPPOCAMPUS OF THE RAT 24 HOURS AFTER SOCIAL DEFEAT

Area	Control	Defeat	<i>P</i>
CA1 stratum oriens	1.01 ± 0.07	1.09 ± 0.14	0.44 <sup>N.S.</sup>
CA1 stratum radiatum	1.02 ± 0.01	1.14 ± 0.12	0.36 <sup>N.S.</sup>
CA3 stratum oriens	0.99 ± 0.04	1.26 ± 0.10	0.04*
CA3 stratum radiatum	1.01 ± 0.03	1.28 ± 0.08	0.006*

\* Indicates significantly altered ratio in NMDA/AMPA binding at the 0.05 level.

Multigrade developer), rinsed in distilled water and fixed for 8 min in 1.5 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> · 5H<sub>2</sub>O. Films were analyzed using an automated image analysis system (IBAS) to determine the amount of [<sup>3</sup>H]CNQX and [<sup>3</sup>H]CGP39653 binding in the hippocampus. Optical densities were converted into pmol/mg using the standards present on the corresponding film. Means from total binding (six sections) and non-specific binding (two sections) were calculated per area/animal. Specific binding was measured by subtracting non-specific binding from the total binding. Absolute binding of the ligands for the different areas ± S.E.M. in control animals is presented. Changes in receptor binding are presented as percentage of binding in control rats, and were analysed using the Student's *t*-test (two-tailed). To reveal whether the altered binding after the stress experience results in a dominance of one of the investigated receptors, we calculated the ratio NMDA/AMPA binding in control and stressed rats in different hippocampal areas. Analysis was performed using the Mann-Whitney *U*-test (two-tailed). Significance level was *P* < 0.05.

In control animals, [<sup>3</sup>H]CNQX labeling (Fig. 1) was mainly found in hippocampal CA1 stratum radiatum (CA1r) and CA1 stratum oriens (CA1o), followed by strong labeling in the molecular layer of the dentate gyrus (DG), CA3 stratum radiatum (CA3r) and CA3 stratum oriens (CA3o). [<sup>3</sup>H]CGP39653 was bound approximately 30% less than [<sup>3</sup>H]CNQX (Fig. 1) and labeling was mainly found in CA1 stratum radiatum and stratum oriens, inner molecular layer of the dentate gyrus (DGi), followed by labeling in CA3 stratum oriens, CA3 stratum radiatum and outer molecular layer of the dentate gyrus (DGo). Social stress decreased [<sup>3</sup>H]CNQX binding in CA3 stratum radiatum (Fig. 2a, *P* = 0.09) but enhanced [<sup>3</sup>H]CGP39653 binding (Fig. 2b, *P* = 0.005). The ratio of NMDA/AMPA binding is significantly increased in hippocampal CA3 stratum oriens (Table 1, *P* = 0.04) and CA3 stratum radiatum (*P* = 0.006).

The present study shows that a single unescapable social stress-experience of defeat increases NMDA receptor and decreases AMPA receptor binding specific in CA3 stratum radiatum of male rats. Consequently, the ratio between NMDA and AMPA receptors is significantly increased in CA3 stratum oriens and CA3 stratum radiatum. Although both control and experimental rats receive some handling stress, the observed differences seem to be the result of the stress-experience of defeat since this is the only difference in the experimental procedure.

Since only a single concentration of both ligands was used, it is difficult to conclude whether the alterations are due to the altered number of binding sites (*B*<sub>max</sub>) or to altered affinity for the ligand (*K*<sub>d</sub>). Both stress-induced alterations of *B*<sub>max</sub> and *K*<sub>d</sub> are possible regarding the time course of the effect (24 h) and the presence of allosteric binding sites at both receptors. The effect of the stress, however, seems to be a specific rather than a general effect since an increase in NMDA receptor binding, concomitant with a decreased AMPA receptor binding occurs.

Our results seem to be contradictory to the results of Tocco et al. [21], in that they observed an increase in AMPA-binding in hippocampal CA1 stratum oriens, CA1 stratum radiatum, CA3 stratum radiatum after 1 h severe stress, whereas NMDA binding was not altered. They interpreted their data as an increase in affinity of the AMPA receptor which might result in an increased fast synaptic transmission. The different results, however, might indicate that the effect of stress on glutamate receptor-binding changes in time (receptor investigation 1 vs 24 h after stress), or is stressor specific. Currently, we are investigating the effects of our stress on AMPA and NMDA receptor binding at different times after defeat.

The net amount of the investigated ionotropic receptors seems to be reduced after the single stressor, since the reduction in AMPA binding exceeds the increase in NMDA binding. It is, therefore, interesting that Halpain and McEwen [5] found a decrease in [<sup>3</sup>H]glutamate binding in the hippocampus after several days of corticosterone administration. Activation of AMPA-kainate receptors reduces Mg<sup>2+</sup> blockade of the NMDA receptor channels and allows Ca<sup>2+</sup> to permeate [14]. This Ca<sup>2+</sup> entry triggers several postsynaptic events. The present finding that a single social stress-experience alters the ratio NMDA/AMPA receptor in the CA3 area might therefore have consequences for glutamatergic neurotransmission in this hippocampal region and be involved in the reduced plasticity seen after stress in glutamate-involved events as long-term potentiation [4, 19, 20].

Unescapable stress increased NMDA receptor binding in the hippocampal CA3 stratum radiatum, suggesting

increased impact of glutamate via NMDA receptors in this area. NMDA receptors are involved in the excitotoxic actions of glutamate [12], and their upregulation after stress may have consequences for hippocampal CA3 neurons. Indeed acute and chronic stress can influence the ultrastructure of hippocampal neurons in the CA3 and CA4 area and glucocorticoids seem to be involved in this effect [1, 11, 16, 17, 23–25]. A possible mechanism via which chronic corticosterone administration or stress could adversely affect CA3 pyramidal cells is by altering glutamatergic mossy fiber activity, which directly influences CA3 neurons via the release of glutamate, resulting in an increased  $\text{Ca}^{2+}$  influx [11]. Although the distribution of glucocorticoid receptors in the hippocampus does not explain why the CA3 area is particularly vulnerable [15], the involvement of the NMDA receptor in this exacerbating effect of glucocorticoids is convincing [1]. Recently Watanabe et al. [24] have reported that an anti-epileptic drug, which is known to interfere with excitatory amino acid release and actions, prevents stress- and corticosterone-induced atrophy of hippocampal CA3 pyramidal neurons. Therefore, a synergistic action of glutamate and corticosteroids seems to be involved in stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. In addition to these findings, our results point to a possible role for the NMDA receptor. This is in line with previous findings that both adrenal integrity and NMDA receptors are involved in hippocampal metabolism, as measured by lactate formation, during and after stress [10, 18].

In conclusion, our data suggest that a single unescapable social stress induces specific rather than general altered impact of glutamate on hippocampal CA3 neurons and that these alterations might result in altered functioning of hippocampal CA3 neurons. Future studies will assess the role of time in the stress induced effects and whether the altered glutamate receptor-binding is accompanied by altered morphology, calcium-influx, metabolic activity and free radical generation.

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